STUDIES ON THE INTERACTION OF ZINC AND OTHER BIVALENT METALS WITH THE CONTRACTILE ELEMENT IN GLYCEROL-EXTRACTED MUSCLE

With Special Reference to the Relaxing Effect of Zinc

By

K. A. PAUL EDMAN

Reprinted from

ACTA SOCIETATIS MEDICORUM UPSALIENSIS

Upsala Läkareförenings Förhandlingar

VOL. LXIV, NOS. 1–2, 1959

(pp. 97–110)

STUDIES ON THE INTERACTION OF ZINC AND OTHER BIVALENT METALS WITH THE CONTRACTILE ELEMENT IN GLYCEROL-EXTRACTED MUSCLE

With Special Reference to the Relaxing Effect of Zinc

AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten
vid Uppsala Universitet för vinnande av medicine doktorsgrad
framlägges till offentlig granskning å Farmakologiska
Institutionens föreläsningssal lördagen den 9 maj
1959 kl. 10 f. m.

av

K. A. PAUL EDMAN
Med. lic., Kalm.

UPPSALA 1959
ALMQVIST & WIKSELLS BOKTRYCKERI AB

From the Pharmacological Institute, University of Uppsala, Sweden.

Studies on the Interaction of Zinc and Other Bivalent Metals with the Contractile Element in Glycerol-extracted Muscle

with Special Reference to the Relaxing Effect of Zinc

by

K. A. PAUL EDMAN

The present article comprises a summary of earlier investigations, which have been reported in the following papers:

I. On the contraction of glycerol-extracted muscle fibre bundles under highly isometric conditions, *Acta physiol. scand. 41,* 229 (1957).

II. The effect of zinc and certain other bivalent metal ions on the isometric tension development of glycerol-extracted muscle fibre bundles, *Acta physiol. scand. 43,* 275 (1958).

III. Relaxation and visco-elastic behaviour of glycerol-extracted muscle fibre bundles in the presence of zinc and ATP, *Acta physiol. scand. 45,* 164 (1959).

IV. Relaxation of glycerol-extracted muscle fibre bundles induced by zinc in the presence of ATP and other polyphosphates, *Acta physiol. scand.,* in press (1959).

V. The binding of zinc to the glycerol-extracted muscle, and its relaxing effect, *Acta physiol. scand.,* in press (1959).

In the following these articles will be referred to using the Roman numerals given above.

Introduction

The glycerol-extracted muscle fibre, introduced by Szent–Györgyi (1949), permits a separate study of the contractile element in situ. Contraction can be induced by different nucleoside triphosphates.

9 – 593511 *Acta Societatis Medicorum Upsaliensis* 64: 1–2

R. UNIV. BIBLIOTHEEK LEIDEN

Among these ATP[1] brings about the strongest contraction. It is probable that ATP constitutes a source of energy for the contraction process in the living muscle cell, but it is unclear if this energy is provided to the contractile element directly, as is probably the case in the glycerol-extracted fibre, or through some intermediate link (see reviews by Weber and Portzehl 1954, Fleckenstein 1955, Morales, Botts, Blum and Hill 1955, Buchthal, Svensmark and Rosenfalck 1956, Perry 1956, Gelfan 1958). This uncertainty must be kept in mind in evaluating the results obtained in studies of the reaction between ATP and the glycerol-extracted fibre.

Relaxation of a contracted glycerol-extracted fibre has also become possible within the last few years. Some of the relaxing substances hitherto described are certainly unphysiological, e.g. ethylenediamine tetraacetate (EDTA) (Bozler 1954, Watanabe 1955) and mersalyl (Portzehl 1952), while a protein factor, the "Marsh–Bendall factor", discovered by Marsh (1952) is a normal constituent of skeletal muscle.

Magnesium and calcium are present physiologically in muscle in relatively high concentrations, about 10 and 1 mmoles per kg wet tissue respectively. The effects of these two metals on the contractile system, on the contractility and the ATPase activity, have accordingly been extensively studied. On the other hand, no thorough investigation has, up to the present, been performed with zinc, although the concentration of zinc in skeletal muscle (about 0.8 mmole per kg wet tissue) is of the same order of magnitude as that of calcium. Zinc inhibits the ATP-induced shrinking of precipitated actomyosin (Gerendás 1941) and the semi-isometric contraction of glycerol-extracted muscle fibres (Hasselbach 1953). As demonstrated later (Edman 1956) at strictly isometric recording the zinc ion even at low bath concentrations induces a complete and fully reversible relaxation of previously contracted fibres, when ATP is present in a sufficient concentration. In the present investigation the zinc-induced relaxation has been explored in more detail. For comparison, the effects of certain other bivalent metal ions on the contractility of glycerol-extracted fibres have also been investigated.

Some methodological factors of relevance to the study of glycerol-extracted muscle fibres

ATP is continously dephosphorylated when reacting with the contractile protein. On the other hand, the restitution of ATP is in-

[1] The following abbreviations will be used: ADP, adenosinediphosphate; ATP, adenosinetriphosphate; EDTA, ethylenediamine tetraacetate; ITP, inosinetriphosphate; PP, inorganic pyrophosphate; TPP, inorganic tripolyphosphate; UTP, uridinetriphosphate.

significant in thoroughly extracted fibres, like those used in the present investigation. Because of the continous consumption of ATP, its penetration into the fibres is limited. For instance, as demonstrated by Hasselbach (1952), only a peripheral layer of 1 μ is reached when the bath concentration of ATP is 0.23 mM. This implies that a central nucleus remains inactive during the contraction of the fibre. It is probable that the shortening of the fibre as a whole will be hampered by the passive resistance of the inactive parts. The same interference source must also be important in "isometric" contraction unless the recording is performed at high degree of isometry as in the methods described by Ranney (1954) and Dettli and Bing (1956).

In the present investigation a new method for strict isometric recording has been used, built on the principle of a torsion balance (I). By means of this recording device the deviation from strict isometry during the contraction course could be reduced to 0.2–0.6 percent of the initial fibre length (10 mm). There is a striking difference between the rates of contraction at high-grade isometry and at semi-isometric recording. With the recording technique used in the present investigation a practically constant tension is reached after only 1–2 min with a fibre bundle (approximate cross section: 100×200 μ) contracted by 1.0 mM ATP, while at least 15–20 min are needed for reaching a final tension when a few percent shortening is allowed (Portzehl 1952). It is conceivable that the relative slowness of the semi-isometric contraction results from the viscous resistance of the passive central parts of the fibres.

The penetration of ATP into a fibre bundle is more complicated than the penetration into a single fibre, since it is conceivable that the endomysium, probably lacking in ATPase activity, is more readily penetrated by ATP than the fibres themselves. By studying the time required for reaching a constant isometric tension with a definite ATP concentration but with different thickness of the fibre bundles, conclusions could be drawn as to the depth of diffusion of ATP into a fibre bundle (I). It was shown that ATP in a bath concentration of 0.23 mM probably penetrates about 40 μ into the fibre bundle. It was also demonstrated that this diffusion depth is probably reached via the endomysium, while the single fibres are penetrated only into a depth of 1 μ, a finding in good agreement with those of Hasselbach (1952).

Since a fibre bundle is not activated throughout by ATP, the tension developed during contraction may be expected to be proportional to the circumference of the bundle and not to the total cross section area. This was demonstrated (I) to hold true for fibre bundles of the thickness used in this investigation, i.e. with a cross section area less than about 200×300 μ.

There is also a diffusion problem for ATP outside the fibre bundle, even when, as in the present investigation, ATP is most effectively

added by exchanging the vessel with the original bath solution for another containing the ATP solution. A boundary layer of the original solution remains around the fibre bundle and this layer must accordingly be penetrated by ATP before ATP reaches the fibre bundle surface. The latencies, the times from addition of ATP to the start of the contraction, obtained with different ATP concentrations are compatible with the existence of an unstirred boundary layer 30–45 μ in thickness (I).

Effects of some bivalent metal ions on the ATP-induced isometric contraction, and relaxing effects on previously contracted fibre bundles

In a survey of the effects of certain bivalent metal ions on the isometric contraction of glycerol-extracted fibre bundles (II) some remarkable effects of zinc and calcium were found. In comparatively high concentrations these metals ($\geq$ 0.01 mM zinc and 10 mM calcium) inhibited the entire contraction course. On the other hand, in concentrations one tenth of these they increased the final tension while the initial tension development was inhibited (zinc) or not significantly affected (calcium). Similar effects on the final tension were obtained with mercury and cadmium, i.e. an inhibition with $\geq$ 0.01 mM mercury and $\geq$ 0.1 mM cadmium and a slight increase in the final tension with lower concentrations of the metals.

Since the tension of the whole fibre bundle is determined not only by the tension of the single elements but also by the depth of penetration of ATP into the bundle and the single fibres, it cannot be concluded from these results whether the metals have a qualitatively different action on the single *contractile element* at high and low concentrations. As has been pointed out (II), both the decrease in final tension of the fibre bundle obtained with high concentrations of the metals and the increase in final tension with lower metal concentrations are compatible with the concept of a decrease in tension of the single contractile element, if there is a simultaneous inhibition of the ATPase activity. The decrease in tension of the single element will then be compensated to a certain degree by deeper penetration of ATP into the bundle and the single fibres made possible by inhibition of the ATPase activity; it is conceivable that a certain decrease in tension of the single element may even be overcompensated by deeper penetration of ATP.

According to Hasselbach (1953) and Hasselbach and A. Weber (1955), the organic mercury compound mersalyl is the only one of several ions and compounds of heavy metals studied which produces

relaxation of contracted fibres and fibre bundles in the presence of ATP. However, under suitable conditions (II), e.g. in the presence of 0.4 mM ATP and 1 mM magnesium, it is possible to induce relaxation with several bivalent metals (zinc, cadmium, mercury, calcium, cobalt and manganese). The metal concentrations needed in the bath for induction of complete isometric relaxation at these ATP and magnesium concentrations are 0.025 mM for zinc, about 1.0 mM for cadmium and 0.01–0.1 mM for mercury; with calcium, cobalt and manganese in a concentration as high as 10 mM only partial relaxation is obtained.

Calcium (10 mM), strontium (10 mM), and mercury (0.1 mM) induce partial relaxation of a contracted fibre bundle also in the absence of ATP. The relaxing effect of calcium in the concentration mentioned is even greater in the absence of ATP than in the presence of 0.4 mM ATP. Similarly, the relaxation with 10 mM strontium is evident in the absence of ATP, but no effect is obtained in the presence of 0.4 mM ATP. On the other hand, zinc, even in the highest concentration studied (0.88 mM), is unable to induce relaxation when tested in the absence of ATP. Higher concentrations of zinc were not investigated in order not to exceed the solubility product for $Zn(OH)_2$. Cadmium in 1 mM concentration is also without effect in the absence of ATP; with 5 mM cadmium there is an insignificant decrease in tension.

After relaxation with mercury the contractility cannot be restored by washing with ordinary mercury-free bath solution. The relaxation induced with the other metals mentioned is more or less reversible. Zinc seems to be the most interesting metal inducing relaxation. Its effect is evident even at low bath concentrations, and, the relaxation is completely reversible by mere washing with zinc-free bath solution. At least 14 contraction-relaxation cycles may be produced in the same fibre bundle without any notable change in contractility.

Zinc-induced relaxation

The relaxation induced by the Marsh–Bendall factor (Hasselbach and Weber 1953, Weber and Portzehl 1954) and EDTA (Watanabe 1955, Watanabe and Sleator 1957) needs an ATP concentration as high as 2–5 mM. This is in striking contrast to the situation in the zinc + ATP-induced relaxation (IV). Partial relaxation is evident with 0.1 mM zinc in the presence of 0.006 mM ATP and complete relaxation in the presence of 0.05–0.4 mM ATP. By further increase in the ATP concentration the relaxing effect decreases. For reaching this effect, "superoptimal" with respect to relaxation, higher concentrations of ATP are needed the higher the zinc concentrations used.

Conclusions as to the mechanism of the inhibition of relaxation at superoptimal ATP concentrations could be drawn by determination of the ATP concentrations needed for obtaining a definite tension in the fibre bundle at varied zinc concentrations. It was demonstrated (IV) that the inhibition of relaxation is probably due to the complex formation between zinc and ATP in the bath. Several observations (II, IV) have made it probable that the relaxing effect of zinc is dependent upon an accumulation of zinc in the fibre protein. Thus, with an adequate increase in the ATP concentration the zinc ion concentration in the bath drops to such an extent through the complex formation between zinc and ATP that the accumulation of zinc in the fibre protein becomes insufficient for relaxation.

The same tension level is reached for a particular zinc + superoptimal ATP concentration irrespective of whether the previous tension is higher or lower. This shows that the changes induced by zinc + ATP in the contractile system are fully reversible in agreement with the good repeatability of the contraction-relaxation cycle induced with zinc + ATP, as mentioned above. It is obvious from this finding that effects on the final tension of the zinc + ATP-induced relaxation may equally well be studied by recording the final tension of the contraction as done in certain series of experiments (IV, V).

With zinc in the presence of a low ATP concentration (0.006–0.02 mM) it is possible to relax a contracted fibre bundle to a certain level only, but it is not possible, on the other hand, to reach the same tension level by contracting a fibre bundle that has a lower tension previously. This is understandable (IV), since even in the absence of zinc, ATP in concentrations below 0.02 mM is unable to induce contraction under the conditions used here.

UTP and ITP may to a certain degree replace ATP as contraction-inducing agent. It is also possible to induce isometric relaxation with zinc in the presence of UTP and ITP instead of ATP (IV), e.g. in the following combinations: 0.2 mM zinc + 1 mM magnesium + 0.4 mM UTP, ITP or ATP. Inorganic pyrophosphate (PP) and triphosphate (TPP) cannot induce contraction but are, on the other hand, able to relax previously contracted fibres. The zinc ion does not change the reactivity of the fibres to PP and TPP (0.25–1.0 mM). These results are compatible with the theory advanced by the Weber school (Weber and Portzehl 1952, 1954, Weber 1955, 1958) and lately by Bendall (1958 b) that the effect of the relaxant, in their case mersalyl, EDTA and the Marsh–Bendall factor, would be a mere inhibition of the contractility, thereby permitting the nucleosidetriphosphate to act only as a relaxing agent like the inorganic polyphosphates. It should be noted, however, that the relaxing effect of zinc + ATP may also be obtained with ATP concentrations ($\geq$ 0.006 mM), which are lower than the threshold concentration (0.02 mM) needed to induce iso-

metric contraction. With such low ATP concentrations it would consequently seem unnecessary to inhibit the contractility with zinc, but yet, zinc is needed for obtaining a relaxing effect (IV). However, this may be explained by the fact that with intact ATPase activity ATP cannot reach sufficiently far into the bundle and will not be able to relax the deeper layers contracted at a higher ATP concentration. In the presence of zinc, on the other hand, the ATPase activity is completely inhibited (IV) thereby allowing ATP to penetrate the bundle throughout.

Magnesium stimulates the ATP-induced contraction with optimal effect at 1–10 mM bath concentration (II, IV). Reduction of the magnesium concentration in an isometrically contracted fibre bundle at unchanged ATP concentration decreases the tension, but it is not possible to relax the fibre bundles completely in this way. Addition of zinc, on the other hand, brings about complete relaxation. The antagonism between the zinc and magnesium effects was directly demonstrated by studying the inhibition of the contractility in fibre bundles induced by zinc at varied magnesium concentrations. It was found (IV) that a zinc concentration (0.0076 mM) almost completely abolishing the isometric contraction by 0.24 mM ATP at 0.01 mM magnesium is practically without effect when the magnesium concentration is increased to 1 mM. However, the experimental data show that the effect of zinc is probably not due to a simple competition with magnesium.

Calcium in a comparatively high concentration, 10 mM, increases the rate of the relaxation induced with zinc (0.2 mM) + ATP (0.4 mM). This is in accordance with the finding that calcium itself in that concentration brings about isometric relaxation of a contracted fibre bundle. The calcium effect is in marked contrast to the situation in relaxation with EDTA (Bozler 1954, Watanabe 1955) and the Marsh–Bendall factor (Marsh 1952, Bendall 1953), where calcium, even in low concentrations (0.5 mM), inhibits the relaxation.

The rate of the isometric relaxation induced with zinc + ATP is limited by the rate of accumulation of zinc in the bundles (II, Fig. 3). In fibre bundles pretreated with zinc subsequent to the contraction the relaxation is relatively rapid; the half time for reaching complete isometric relaxation with 0.2 mM zinc + 1 mM magnesium + 0.4 mM ATP in such pretreated bundles is 2–3 sec. The relaxation rate is not markedly changed by using a high ATP concentration, e.g. 2 mM zinc + 1 mM magnesium + 5 mM ATP (according to the foregoing the zinc concentration must be increased to a corresponding degree if relaxation is to be obtained with zinc at higher ATP concentration).

Also isotonic relaxation is obtained with zinc + ATP. However, at isotonic recording the relaxing effect of zinc + low concentration of ATP (0.4 mM) is markedly weaker than that obtained with zinc +

high concentration of ATP (5 mM). For instance, at a load of about 250 g/cm², 0.2 mM zinc + 1 mM magnesium + 0.4 mM ATP induces complete relaxation only if the previous shortening does not exceed 25 percent of the initial fibre length: at 75 percent shortening no relaxation is obtained. At the same load, however, the combination 2 mM zinc + 1 mM magnesium + 5 mM ATP is able to cause complete relaxation even after 60 percent previous shortening of the fibre bundle. Complete relaxation of 60 percent shortened fibre bundles was also obtained with 4 mM EDTA + 4 mM magnesium + 4 mM ATP as previously demonstrated by Watanabe and Sleator (1957).

In order to test if the differences in relaxing effect of zinc + ATP at isotonic and isometric recording may be due to a different need for increased visco-elastic compliance, a study of the passive visco-elastic properties of the fibre bundles was performed.

Visco-elastic behaviour of glycerol-extracted muscle fibres

Beside its contraction-inducing effect ATP in comparatively high concentrations (~ 1–10 mM) decreases the passive resistance to stretch of glycerol-extracted fibres. This rigidity-lowering effect of ATP has previously been studied by the Weber school (Portzehl 1952, Weber and Portzehl 1952, 1954, Hasselbach 1956) and has been called "plasticising" action. As a measure of the "plasticising" effect of ATP changes in the static modulus of elasticity have been used by the Weber school. However, a change in the static elasticity does not give any complete picture of the effect of a substance on the passive visco-elastic properties of a glycerol-extracted fibre. In the present investigation (III, IV) the fibre bundles after having been equilibrated with a certain bath solution were subjected to a quick stretch and the tension course was recorded continously until an equilibrium level was reached.

For evaluation of the results a model for the passive visco-elastic properties of the muscle fibre was used consisting of an elastic element in series with another elastic element which is shunted by a viscous element. The effects on the different elements were calculated. It was found that zinc + high ATP concentration (2 mM zinc + 1 mM magnesium + 5 mM ATP) increases the compliance of the elastic elements and also decreases the viscous resistance. Similar effects were obtained with 0.2 mM zinc + 1 mM magnesium + 0.4 mM *ITP* and even more pronounced effects were obtained with 4 mM EDTA + 4 mM magnesium + 4 mM ATP. On the other hand, with zinc + low ATP concentration (0.2 mM zinc + 1 mM magnesium + 0.4 mM ATP) the visco-elastic properties of the fibre bundles are not significantly changed.

According to the hypothesis advanced by H. H. Weber (Weber and

Portzehl 1952, 1954, Weber 1955, 1958), the relaxation induced with a relaxing factor in the presence of ATP is due to the "plasticising" effect of ATP. However, it is evident from the visco-elasticity measurements presented in this work that no marked change in the visco-elastic properties is needed in order to get a complete relaxation of glycerol-extracted fibres at strict isometric recording. As mentioned above, the combination 0.2 mM zinc − 1 mM magnesium − 0.4 mM ATP induces complete isometric relaxation although there is no significant visco-elastic change. Moreover, the tension decrease caused by this zinc − ATP-combination is not less pronounced than that obtained with the combinations 2 mM zinc − 1 mM magnesium − 5 mM ATP (III), 0.2 mM zinc − 1 mM magnesium − 0.4 mM ITP (IV) and 4 mM EDTA − 4 mM magnesium − 4 mM ATP (III), which all markedly decrease the visco-elastic resistance. It was also demonstrated by experiments (III) that the rate of isometric relaxation is not limited by visco-elastic factors. Thus, relaxation seems to be a process quite different from a mere change in visco-elasticity.

The need for increased visco-elastic compliance is probably different in isometric and isotonic relaxation. At strict isometry even at the submicroscopic level, the relaxation would be the mere disappearance of a force, which would not need any change in visco-elasticity. On the other hand, if a series elastic component is present, there is a shortening of the contractile unit, even though the fibre bundle as a whole is kept at strict isometry during contraction. It is probable that the disappearance of this shortening would be dependent on the visco-elastic resistance. The need for increase in visco-elastic compliance would be greatest in relaxation from maximum isotonic shortening. The fact that no manifest change in visco-elastic resistance is needed for *isometric* relaxation therefore indicates (III) that the isometric relaxation of glycerol-extracted fibres is mainly the disappearance of a force, i.e. the shortening of the contractile unit is insignificant during an isometric contraction induced with 0.4 mM ATP as in the present experiments. The greater need for visco-elastic compliance at *isotonic* relaxation is also evident from the present investigation. With the zinc − ATP-combination, which does not give any visco-elastic change, the relaxation is insignificant at extremely shortened fibres, while, under the same conditions, the relaxation is complete if a zinc − ATP-combination is used which markedly lowers the visco-elastic resistance. For the role of the inactive central parts of the bundle and the single fibres during isotonic relaxation, see Discussion in III.

The binding of zinc to muscle tissue

Voluntary muscle of vertebrates contains zinc in a rather high concentration. In analyses performed on rabbit psoas (V) 3.2 mmoles

zinc were found per kg dry weight, in good agreement with the results reported by others (Koga 1934, Vallee and Altschule 1949, Hamm 1955, Koch, Smith, Shimp and Connor 1956). The calcium concentration is of the same order of magnitude, viz. 3.6 mmoles, while the magnesium concentration is 15 times higher, viz. 46.5 mmoles per kg dry weight.

After glycerol-extraction and thorough washing with a 100 mM potassium chloride solution (pH 7.3) the muscle tissue still contains zinc in a rather high concentration, 2.4 mmoles per kg dry weight. However, if the simultaneous weight loss of the tissue (about 30 percent) is taken into account, it follows that approximately 2 mmoles zinc are lost per kg dry glycerol-extracted muscle tissue during the extraction, which corresponds to about 50 percent of the zinc content originally present.

The zinc ion has a great affinity for the fibre proteins. After incubation with 0.0025 mM zinc (pH 7.3), which is the minimum concentration of free zinc ions necessary for complete relaxation, the concentration in the muscle tissue is about 6 mmoles per kg dry weight as opposed to 2.4 mmoles before the incubation. Thus, the amount of zinc, 3–4 mmoles, that has to be taken up by the fibres for full relaxing effect, is close to the amount, about 2 mmoles, that is lost during the extraction.

The glycerol-extracted fibre tissue is able to accumulate zinc in concentrations far exceeding the threshold concentration needed for total relaxing effect; the tissue is unsaturated even at 1 mM zinc concentration in the medium.

The same pH-dependence is obtained for the contraction-inhibiting effect of zinc as for the binding of zinc in excess to the fibres; in both cases groups with a pK-value of about 7 are evidently involved. As has been pointed out (V), the findings are compatible with coupling of the zinc ion to imidazole side chains in the protein.

There is an additive synergism of the contraction-inhibiting effects of zinc and mersalyl, which implies that zinc and mersalyl probably act on the same sites. In view of the high affinity of mercury for SH-groups, as demonstrated with small molecules (Stricks, Kolthoff and Heyndrickx 1954), it is conceivable that SH-groups may be involved in the functionally relevant coupling of mersalyl, and, consequently, also in the functionally relevant coupling of zinc to the contractile protein. However, as pointed out (V), a specific binding of mercury to protein molecules may occur without involvement of SH-groups. Anyhow, the pH-dependence of the zinc effect shows that if SH-groups at all partake in the functionally significant binding of zinc, other groups, probably imidazoles, must also be involved.

Comparison of the relaxing effects obtained with zinc and the Marsh-Bendall factor

The so called Marsh–Bendall factor represents the relaxing activity of a muscle extract in the presence of magnesium and ATP. The factor has not yet been completely defined. According to recent reports of Kumagai, Ebashi and Takeda (1955), Ebashi (1957, 1958), Lorand, Molnar and Moos (1957) and Gergely, Briggs and Kaldor (1958) the factor activity seems to depend on more than one component.

Relaxing effects on glycerol-extracted fibres have been reported with ATP in the presence of several ATP-restituting enzyme systems, viz. myokinase (Bendall 1954), creatinephosphate-creatinephosphokinase (Bozler 1953, Goodall and Szent-Györgyi 1953) and phosphoenolpyruvate-pyruvatephosphokinase (Moos and Lorand 1957). However, no relaxation is obtained with these enzymes in exhaustively extracted fibres. According to Portzehl (1957), Briggs and Portzehl (1957) and Bendall (1958 a) the relaxing effect is dependent on the presence of the Marsh–Bendall factor; the ATP-restituting enzyme systems would, according to these authors, only make it possible to reach an ATP concentration inside the fibres sufficiently high for relaxation with the remaining factor activity. On the other hand, Ebashi (1957, 1958) and Lorand, Molnar and Moos (1957, see also discussion in Ebashi 1957) have presented results suggesting that ATP-restituting enzyme systems may constitute an integral part of the so called Marsh–Bendall factor.

The zinc ion does evidently not induce its relaxing effect over the Marsh–Bendall factor. The effects of magnesium and calcium on the zinc-induced relaxation are quite different from those obtained in the relaxation with muscle extract. In the zinc-induced relaxation considerably lower ATP concentrations are needed, and furthermore, ATP may be replaced by ITP and UTP in contrast to the situation with the Marsh–Bendall factor. The relaxing effect of zinc is not markedly influenced by changes in extraction time of the fibres, which further stresses its independence of the Marsh–Bendall factor.

As well as the Marsh–Bendall factor, zinc is a normal constituent of the skeletal muscle cell. It is of interest to note that the zinc uptake required by the glycerol-extracted fibres for complete relaxing effect is close to the amount of zinc lost during the glycerol-extraction. A physiological evaluation of these analytical data is not yet possible. Considering the similarity of the zinc concentration required for relaxation in glycerol-extracted fibres and the concentration of zinc in the intact skeletal muscle cell, it does not seem unreasonable to assume that zinc plays a physiologic role in the contraction-relaxation process.

Acknowledgements

To Professor Ernst Bárány, head of the Pharmacological Institute, University of Uppsala, I wish to express my deep gratitude for his advice and stimulating interest and for all facilities put at my disposal during this investigation.

Professor Fritz Buchthal and Dr. Poul Rosenfalck of the Institute of Neurophysiology, University of Copenhagen, have kindly read several of the manuscripts and given helpful criticism, for which I am sincerely grateful.

I wish to thank Professor Folke Nydahl of the Analytical Department, University of Uppsala, for his advice and for working facilities in the metal analysis studies.

I am also grateful to Docent Bo Malmström of the Biochemical Institute, University of Uppsala, for his interest and advice.

My thanks are also due Professor Gunnar Blix and Professor Gunnar Ågren of the Institute of Medical Chemistry, University of Uppsala, for many working facilities placed at my disposal during the course of the investigation.

I am indebted to the staff of the Pharmacological Institute for their kindness and support. In particular, I wish to thank Mrs. Birgit Andersson for her skilled technical assistance and her unsparing work, Mr. Manne Fredriksson for photographic copying and several other services, Mr. Folke Högberg for building the devices for isotonic and isometric recording, Mr. Claes-Iwar Sjöberg for building and keeping up the electrical equipment, and Mrs. Ann-Mari Kjellberg for her indefatigability in producing new copies of the manuscripts.

For revision of the English text I am indebted to Mrs. Virginia Uhlemann.

The work was supported by grants from the Medical Faculty of the University of Uppsala, the Magnus Bergvall Foundation, Svenska Nationalföreningen mot Tuberkulos and the Medical Research Fund of Svenska Personal-Pensionskassan.

References

Bendall, J. R., Further observations on a factor (the 'Marsh' factor) effecting relaxation of ATP-shortened muscle-fibre models, and the effect of Ca and Mg ions upon it. *J. Physiol. (Lond.) 121*, 232–254 (1953).

Bendall, J. R., The relaxing effect of myokinase on muscle fibres; its identity with the 'Marsh' factor. *Proc. Roy. Soc. B. 142*, 409–426 (1954).

Bendall, J. R., Muscle-relaxing factors. *Nature 181*, 1188–1190 (1958 a).

Bendall, J. R., Relaxation of glycerol-treated muscle fibers by ethylenediamine tetraacetate. *Arch. Biochem. Biophys. 73*, 283–285 (1958 b).

Bozler, E., The role of phosphocreatine and adenosinetriphosphate in muscular contraction. *J. gen. Physiol. 37*, 63–70 (1953).

Bozler, E., Relaxation in extracted muscle fibers. *J. gen. Physiol. 38,* 149–159 (1954).

Briggs, F. N., and Portzehl, H., The influence of relaxing factor on the pH dependence of the contraction of muscle models. *Biochim. biophys. Acta 24,* 482–488 (1957).

Buchthal, F., Svensmark, O., and Rosenfalck, P., Mechanical and chemical events in muscle contraction. *Physiol. Rev. 36,* 503–538 (1956).

Dettli, L., and Bing, R. J., Contractile properties of actomyosin threads and bands prepared from dogs' hearts. *Am. J. Physiol. 187,* 145–150 (1956).

Ebashi, S., Kielley-Meyerhof's granules and the relaxation of glycerinated muscle fibers. *Abstract of Conference on the Chemistry of Muscular Contraction, Tokyo* (1957).

Ebashi, S., A granule-bound relaxation factor in skeletal muscle. *Arch. Biochem. Biophys. 76,* 410–423 (1958).

Edman, K. A. P., Reversible, isometric relaxation of glycerol-extracted muscle fibers by zinc and calcium in the presence of ATP. *Acta Soc. med. Upsal. 61,* 215–220 (1956).

Fleckenstein, A., Der Kalium–Natrium–Austausch als Energieprinzip in Muskel und Nerv. Springer-Verlag, Heidelberg (1955).

Gelfan, S., Muscle. *Ann. Rev. Physiol. 20,* 67–96 (1958).

Gerendás, M., Technisches über Myosinfäden nebst einigen Beobachtungen über ihre Kontraktion. Stud. Inst. med. Chem., Univ. Szeged *1,* 47–57 (1941–42).

Gergely, J., Briggs, F. N., and Kaldor G., Resolution of the relaxing factor of muscle into two components. Abstr. Int. Congr. Biochem., Vienna (1958).

Goodall, M. C., and Szent-Györgyi, A. G., Relaxing factors in muscle. *Nature 172,* 84–85 (1953).

Hamm, R., Über die Bestimmung von Calcium, Magnesium, Zink und Eisen in tierischem Gewebe. *Biochem. Z. 327,* 149–162 (1955).

Hasselbach, W., Die Diffusionskonstante des Adenosintriphosphats im inneren der Muskelfaser. *Z. Naturforschung 7 b,* 334–337 (1952).

Hasselbach, W., Giftwirkungen auf den Arbeitszyklus des Fasermodells. *Z. Naturforschung 8 b,* 212–214 (1953).

Hasselbach, W., Die Wechselwirkung verschiedener Nukleosidtriphosphate mit Aktomyosin im Gelzustand. *Biochim. biophys. Acta 20,* 355–368 (1956).

Hasselbach, W., and Weber, A., Models for the study of the contraction of muscle and of cell protoplasm. *Pharmacol. Rev. 7,* 97–117 (1955).

Hasselbach, W., and Weber, H. H., Der Einfluss des MB-Faktors auf die Kontraktion des Fasermodells. *Biochim. biophys. Acta 11,* 160–161 (1953).

Koch, H. J., Smith, E. R., Shimp N. F., and Connor, J., Analysis of trace elements in human tissues. *Cancer 9,* 499–511 (1956).

Koga, A., Répartition du zinc dans les organes des animaux. Keijo *J. Med. 5,* 97–105 (1934).

Kumagai, H., Ebashi S., and Takeda, F., Essential relaxing factor in muscle other than myokinase and creatine phosphokinase. *Nature 176,* 166 (1955).

Lorand, L., Molnar, J., and Moos, C., Biochemical studies of relaxation in glycerinated muscle. *Biol. Bull. 113,* 323–324 (1957).

Marsh, B. B., The effects of adenosine triphosphate on the fibre volume of a muscle homogenate. *Biochim. biophys. Acta 9,* 247–260 (1952).

Moos, C., and Lorand, L., Studies on the biochemistry of contraction and relaxation in glycerinated muscle. The effects of phosphoenolpyruvate. *Biochim. biophys. Acta 24,* 461–474 (1957).

Morales, M. F., Botts, J., Blum, J. J., and Hill, T. L., Elementary processes in muscle action: An examination of current concepts. *Physiol. Rev. 35,* 475–505 (1955).

Perry, S. V., Relation between chemical and contractile function and structure of the skeletal muscle cell. *Physiol. Rev. 36,* 1–76 (1956).

PORTZEHL, H., Der Arbeitszyklus geordneter Aktomyosinsysteme (Muskel und Muskelmodelle). *Z. Naturforschung 7 b,* 1–10 (1952).

PORTZEHL, H., Bewirkt das System Phosphokreatin-Phosphokinase die Erschlaffung des lebenden Muskels? *Biochim. biophys. Acta 24,* 474–482 (1957).

RANNEY, R. E., A myograph for measurement of the isometric tensions developed by extracted muscle fibers. *J. Appl. Physiol. 6,* 513–516 (1954).

STRICKS, W., KOLTHOFF I. M., and HEYNDRICKX, A., Formation and properties of various mercuric mercapto thioglycolates formed in reactions between mercuric mercury and thioglycolic acid. *J. Am. chem. Soc. 76,* 1515–1519 (1954).

SZENT-GYÖRGYI, A., Free-energy relations and contraction of actomyosin. *Biol. Bull. 96,* 140–161 (1949).

VALLEE, B. L., and ALTSCHULE, M. D., Zinc in the mammalian organism, with particular reference to carbonic anhydrase. *Physiol. Rev. 29,* 370–388 (1949).

WATANABE, S., Relaxing effects of EDTA on glycerol-treated muscle fibers. *Arch. Biochem. Biophys. 54,* 559–562 (1955).

WATANABE, S., and SLEATOR, W., EDTA relaxation of glycerol-treated muscle fibers, and the effects of magnesium, calcium and manganese ions. *Arch. Biochem. Biophys. 68,* 81–101 (1957).

WEBER, H. H., The link between metabolism and motility of cells and muscles. *Symp. Soc. exp. Biol.* (Univ. Press, Cambridge) *9,* 271–281 (1955).

WEBER, H. H., The motility of muscle and cells, Harvard Univ. Press. (1958).

WEBER, H. H., and PORTZEHL, H., Kontraktion, ATP-Cyclus und fibrilläre Proteine des Muskels. *Ergebn. Physiol. 47,* 369–468 (1952).

WEBER, H. H., and PORTZEHL, H., The transference of the muscle energy in the contraction cycle. *Progr. Biophys. 4,* 60–111 (1954).

ALMQVIST & WIKSELL 593511